

Title: Polynucleotide Sequence Encoding Cysteine Protease for Modulation
of Coffee Flavour Precursor Levels In Green Coffee Grains (*as modified*)
Inventor: McCarthy, et al.
App. No.: 10/559,986
Docket No.: 112701-667
REPLACEMENT DRAWING

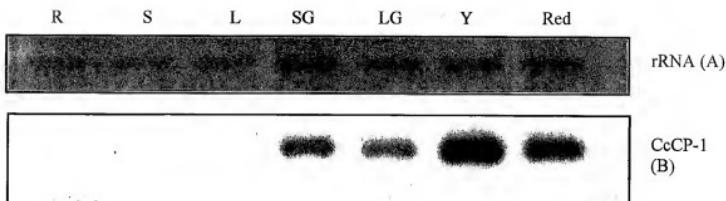


Figure 1: Northern blot analysis of the expression of the cysteine proteinase (CcCP1) gene in different tissues of *Coffea arabica*.

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R S L F SG(g) SG(p) LG(g) LG(p) Y(g) Y(p) Red(g) Red(p)



Figure 2A: Northern blot analysis of the expression of the Cysteine proteinase CcCP-1 gene in different tissues of *Coffea arabica*.

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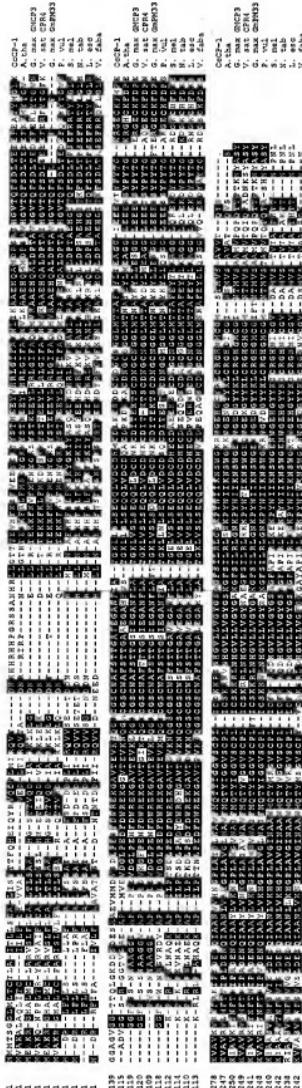


Figure 2B: Alignment of the full sequence of the protein encoded by CcCP-1 cDNA with other full-length cysteine proteases available in the NCBI database.

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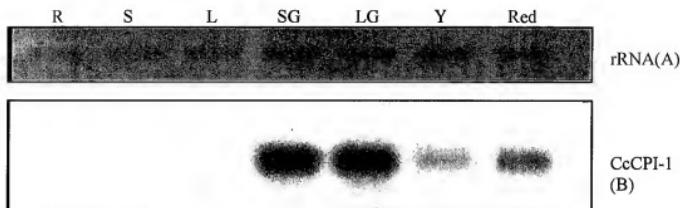


Figure 3: Northern blot analysis of the expression of the cysteine proteinase inhibitor (CcCPI-1) gene in different tissues of *Coffea arabica*.

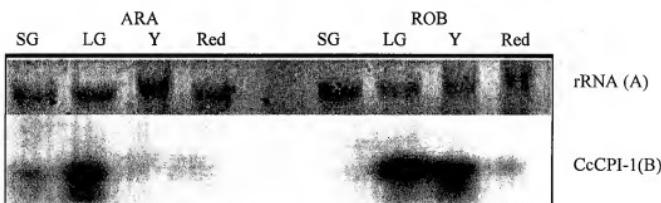


Figure 4: Northern blot analysis of the expression of the cysteine proteinase inhibitor gene (CcCPI-1) at different cherry development stages for *Coffea arabica* (ARA) and *Coffea canephora* (ROB).

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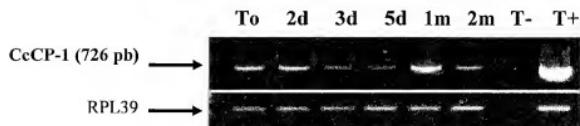


Figure 5. RT-PCR analysis of the expression of *CcCP-1* during *Coffea arabica* grain germination.

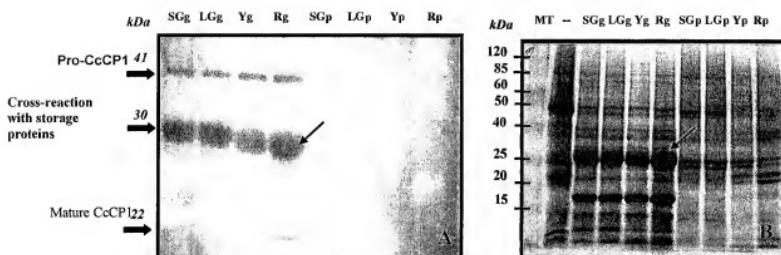


Figure 6A: Western-blot analysis of the expression of CcCP1 protein (Å).

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CPI-1 (SEQ ID NO: 56)
M. dosmest (SEQ ID NO: 57)
sunflower (SEQ ID NO: 58)
R. obtusif (SEQ ID NO: 59)
A. thaliana (SEQ ID NO: 60)

Figure 6B: Optimal alignment of the complete protein encoded by CcCPI-1 cDNA with other homologous full-length cysteine proteinases available in the NCBI database.

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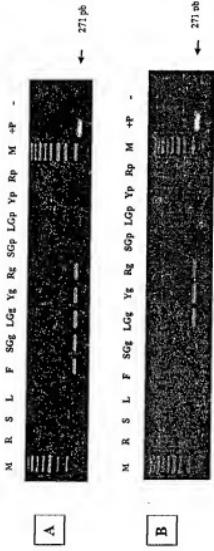


Figure 7: RT-PCR analysis of the expression of CcCP1-1 gene in different tissues of *Coffea arabica* CCCA2 (A) and *Coffea robusta* FRT-32 (B).

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| | | |
|---|---|-----------------|
| M-A-K-V-G-G-I-S-E-S-G-O-N | F-N-S-E-L-E-S-T-L-A-K-E-A-Y-D-D-Y-N-K-O-N-A-L-E' | C-C-F-2 |
| M-A-T-V-G-G-I-Q-K-S-E-S | F-N-S-E-L-E-S-T-L-A-K-E-A-Y-D-D-Y-N-K-O-N-A-L-E' | R. obesinibus |
| M-A-T-V-G-G-I-Q-K-S-E-S | F-N-S-E-L-E-S-T-L-A-K-E-A-Y-D-D-Y-N-K-O-N-A-L-E' | D. caryophyllea |
| M-A-T-L-Q-G-I-K-E-N-E-S | F-N-S-E-L-E-S-T-L-A-K-E-A-Y-D-D-Y-N-K-O-N-A-L-E' | M. esculenta |
| O-F-O-K-V-I-N-S-K-E-Q-V-V-A-G-T-V-Y-Y-L-T-E-V-K-D-G-N-E-K-K-L-Y-E-A-K-V-W-K-P | O-F-O-K-V-I-N-S-K-E-Q-V-V-A-G-T-V-Y-Y-L-T-E-V-K-D-G-N-E-K-K-L-Y-E-A-K-V-W-K-P | C-C-F-2 |
| O-F-S-K-V-N-T-K-E-Q-V-V-A-G-T-V-Y-Y-L-T-E-V-K-D-G-N-E-K-K-L-Y-E-A-K-V-W-K-P | O-F-S-K-V-N-T-K-E-Q-V-V-A-G-T-V-Y-Y-L-T-E-V-K-D-G-N-E-K-K-L-Y-E-A-K-V-W-K-P | R. edentulus |
| O-F-O-K-V-I-N-S-K-E-Q-V-V-A-G-T-V-Y-Y-L-T-E-V-K-D-G-N-E-K-K-L-Y-E-A-K-V-W-K-P | O-F-O-K-V-I-N-S-K-E-Q-V-V-A-G-T-V-Y-Y-L-T-E-V-K-D-G-N-E-K-K-L-Y-E-A-K-V-W-K-P | D. caryophyllea |
| O-W-L-N-F-K-E-V-Q-E-F-K-P | O-W-L-N-F-K-E-V-Q-E-F-K-P | M. esculenta |
| O-W-M-N-F-K-E-V-Q-E-F-K-P | O-W-M-N-F-K-E-V-Q-E-F-K-P | |
| O-W-L-N-F-K-E-V-Q-E-F-K-P | O-W-L-N-F-K-E-V-Q-E-F-K-P | |

Figure 8: Optimal alignment of the complete protein encoded by CcCPI-2 cDNA with other homologous full-length cysteine proteinases available in the NCBI.

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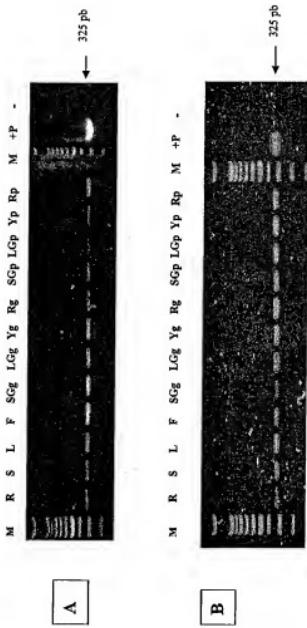


Figure 9: RT-PCR analysis of the expression of CcCPI-2 gene in different tissues of *Coffea arabica* CCCA2 (A) and *Coffea robusta* FRT-32 (B).

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Figure 10: Optimal alignment of the complete protein encoded by CcCPI-3 cDNA with other homologous full-length cysteine proteinases available in the NCBI.

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M A T Y A K S A T T A L S O C Q E K N N V A G G C L S T V P R E S T N P K D P H Y I Q
 M T S K V F U L L E S . S V V L E T P Y A S A A L V G W A S P I D E S N V T D P Q Y V M E
 I L A G E N N X K A G T V W L N V E Y Q P R W D D T T Y M R L A I K T Q D L T C H P
 I G E F A V T N N X K A G T S A L K E F V S Q T O N Y R L A I K T Q D L T C H P
 I S S E Y N V V S Q T O N Y R L A I K T Q D L T C H P
 G T H C D V A L V A E I S E N G T Y S L K W Y N N N N K (SD ID NO: 68)
 I S S E Y N V V S Q T O N Y R L A I K T Q D L T C H P
 G T H C D V A L V A E I S E N G T Y S L K W Y N N N N K (SD ID NO: 70)
 G T H C D V A L V A E I S E N G T Y S L K W Y N N N N K (SD ID NO: 71)

Figure 11: Optimal alignment of the complete protein encoded by CcCP1-4 cDNA with other homologous full-length cysteine proteases available in the NCBI.

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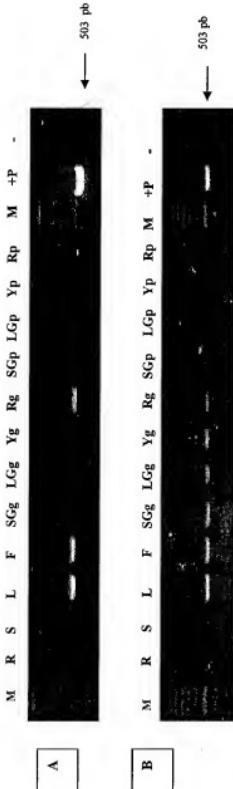


Figure 12: RT-PCR analysis of the expression of CccCP1-4 gene in different tissues of *Coffea arabica* CCC:A2 (Panel A) and of *Coffea robusta* FRT-32 (Panel B)

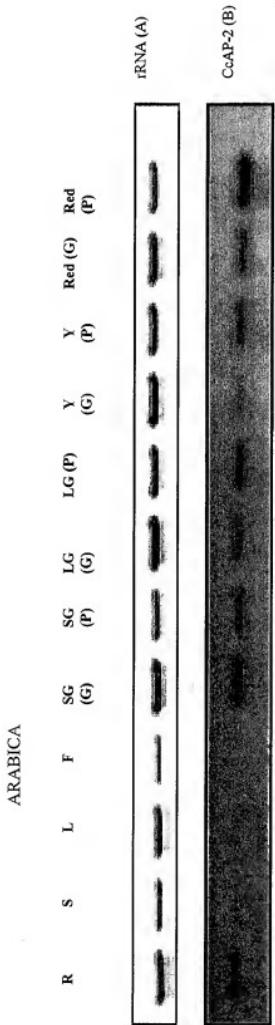


Figure 13: Northern blot analysis of the expression of the aspartic proteinase 2 (CcAP2) gene in different tissues of *Coffea arabica*.

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Figure 14: cDNA sequence and its deduced amino acid sequence of CcCP-4. Lowercase: 5' and 3' non-translated regions; Uppercase: Open reading frame; Bold character: amino acid sequence; *: stop codon.

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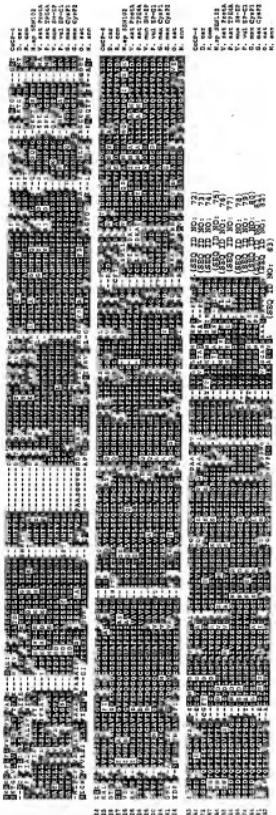


Figure 15: Alignment of the full sequence of the protein encoded by CcC24 cDNA with other full-length cysteine proteases available in the NCBI database

Figure 16. The full length cDNA sequence CcCP-4 KDDL and the partial cDNA sequence CcCP-4 (KDEL).

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CGKAFIAFLAVVAVLIVAMMSITEILRDLASSESLMLYERRSRSHHAWSSRDESEXKREFFENXAKANBHIIHVNOKKXPY
 CGCP-4 KDDI
 CGCP-4 KDEI

-
 -
 -

KLKINSEFADNTNHHFEEPEYPSKSKYFETTMLRGSKINNTGPFHAGKESIAPSVDWERKOGAVTGKVKGKOGSCMFASTTGVVE
 CCCC-4 KDDI
 CCCC-4 KDEI

-
 -
 -

GIRNIKTGGIVLSSEQELDCEIDNEGCGUGLMENAYEIKRKGGGIIYTERLYYKARIGSCDSKNNAPAVTDGHENVF
 CCCC-4 KDDI
 CCCC-4 KDEI

-
 -
 -

ANDENALMAKAVANGPVSVIDAAGSDMQYSSEGYYAGDCCGNLDHGTAVVVGTTAIDGTKXIVKNSWGTGEQGQIR
 CCCC-4 KDDI
 CCCC-4 KDEI

-
 -
 -

ANDENALMAKAVANGPVSVIDAAGSDMQYSSEGYYAGDCCGNLDHGTAVVVGTTAIDGTKXIVKNSWGTGEQGQIR
 CCCC-4 KDDI
 CCCC-4 KDEI

-
 -
 -

GIKIKTGGIVLSSEQELDCEIDNEGCGUGLMENAYEIKRKGGGIIYTERLYYKARIGSCDSKNNAPAVTDGHENVF
 CCCC-4 KDDI
 CCCC-4 KDEI

-
 -
 -

MQRVDAAGGGGCCATAMEASYPLISSHUPKSPSKPDKDEI [SEQ ID NO: 86]
 MQRVDAAGGGGCCATAMEASYPLISSHUPKSPSKPDKDEI [SEQ ID NO: 87]

Decoction 'Decoration #1': Shaded (with solid black) residues that match CcCP-4 KDDI exactly. □

Figure 17. The complete open reading frame of CcCP-4 (KDDI) and the partial open reading frame of CcCP-4 (KDEL).

Figure 17. The complete open reading frame of CcCP-4 (KDDL) and the partial open reading frame of CcCP-4 (KDEL).

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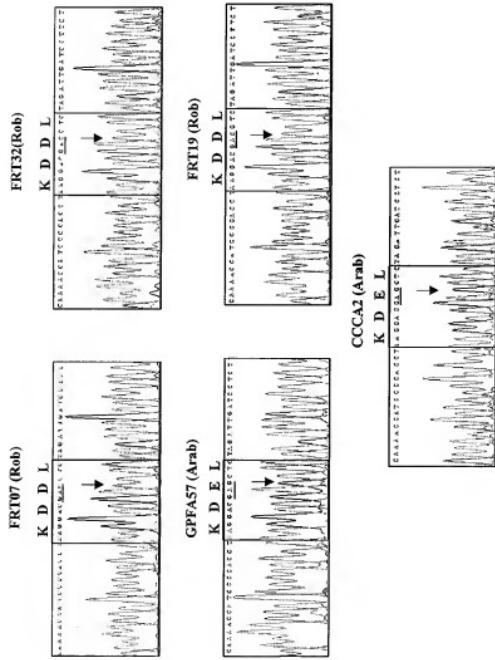


Figure 18: DNA sequence chromatograms for PCR amplified genomic DNA encoding the KDEL/KDDL region of the CcCP-4 gene.

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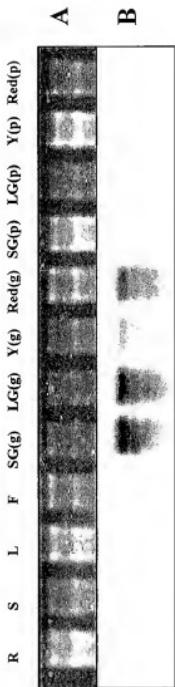


Figure 19. Northern blot analysis of the expression of the Cysteine proteinase CcCP-4 gene in different tissues of *Coffea arabica*.

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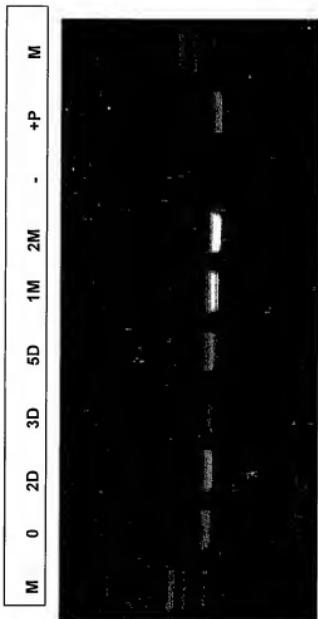


Figure 20. RT-PCR analysis of the expression of CcCP-4 in the whole grain during germination.

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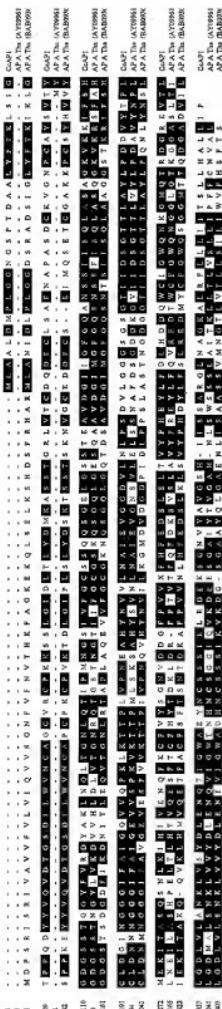


Figure 21: Optimal alignment of the complete protein encoded by CcAP-1 cDNA with other homologous full-length aspartic proteinase sequences available in the NCBI.

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Figure 22: Optimal alignment of the complete protein encoded by CcAP-2 cDNA with other homologous full-length aspartic proteinase sequences available in the NCBI.